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Relative Resistance to Erythromycin in *Chlamydia trachomatis*

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Recent *Chlamydia trachomatis* isolates were tested in a tissue culture system for susceptibility to tetracycline, erythromycin, rosaramicin, rifampin, and clindamycin. Rifampin was the most active drug (minimal inhibitory concentration, ≤0.02 μg/ml). Tetracycline and rosaramicin were highly active, with a concentration of ≤0.25 μg/ml being chlamydicidal. Clindamycin was least active on a weight basis, requiring up to 16 μg/ml to prevent the passage of chlamydiae into a drug-free tissue culture system. Relative resistance to erythromycin was detected; two isolates were capable of limited replication in 1 μg/ml.

Chlamydia trachomatis is recognized as one of the most common of the human sexually transmitted pathogens (8). It is also an important cause of perinatal morbidity in infants. The advent of tissue culture procedures has facilitated isolation of the organism and led, in large part, to the appreciation of its public health import. Because chlamydial infections are readily amenable to chemotherapy, a number of research workers have studied the antibiotic susceptibility of this organism in tissue culture systems (1-4, 6, 7, 9). Although there are some differences in the techniques used, virtually all research workers have come to the same conclusions. Tetracycline is generally considered to be the drug of choice; erythromycin is essentially equivalent to tetracycline in antichlamydial activity. Naturally occurring resistance of chlamydiae to these two antibiotics has never been reported. Different testing procedures make it possible to determine the minimal inhibitory concentration (concentration of antibiotic which produces a 50% reduction in inclusion count) or the minimal chlamydicidal dose (dose which prevents inclusion formation and passage of the organism into a drug-free tissue culture system).

A previous study from this laboratory evaluated the antichlamydial activity of tetracycline, erythromycin, and rifampin against 11 serotypes of *C. trachomatis* (3). In that study, the minimal inhibitory concentrations of tetracycline and erythromycin generally ranged from 0.06 to 0.25 μg/ml, and total suppression of inclusion formation occurred at concentrations of 0.5 to 1.0 μg/ml. Erythromycin was slightly but statistically significantly more active than tetracycline. Rifampin seems to be the most active of the antichlamydial antibiotics (2, 3), but this drug is not routinely used in clinical situations, and resistance can be developed readily in the laboratory (5). Because tetracycline is used routinely to treat chlamydial infections in adults and because erythromycin is the drug of choice in chlamydial infection in infants, we have continued to monitor the antichlamydial activity of these drugs with new isolates. We report herein the detection of relative resistance to erythromycin in some recently isolated *C. trachomatis* strains.

MATERIALS AND METHODS

Assay system. Standard inocula of chlamydial isolates, pretitrated to yield approximately 300 inclusions per cover slip, were used to infect cycloheximide-treated McCoy cells. At 2 h after centrifugation of inoculum into the cells, the medium was replaced with tissue culture medium containing an antibiotic. Each dilution was tested in triplicate, and one tube was used for passage into two new cell monolayers. After centrifugation of passage material, the monolayers were washed several times with medium containing no antibiotic and then incubated for 65 h at 37°C. Inclusion-negative cover slips were stained with Giemsa stain and examined again. Each experiment was repeated twice.

Chlamydial isolates. The chlamydial strains used were six recent isolates. Three had been obtained from the cervix, two had been obtained from infants, and one had been obtained from a throat swab from an adult male. Four were serotyped as DE (10).

Antibiotics. Antibiotics tested included tetracycline hydrochloride, clindamycin, rosaramicin, and erythromycin. Pure powder was dissolved and then diluted in tissue culture medium to yield the final test concentrations. The activity and concentration of the antibiotic solutions were verified by bacterial growth inhibition tests against reference preparations. The highest concentrations of antibiotics were those con-

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centrations that are readily achieved in blood with the usual human dosages.

RESULTS

With these antibiotics, the chlamydicidal levels were never more than one twofold dilution greater than the highest concentration in which any inclusions were found. The duplicate tubes of inclusion-positive cover slips always passed successfully.

The ranges of endpoints observed are presented in Table 1. All chlamydial strains were susceptible to the action of tetracycline (minimal inhibitory concentration, <0.03; minimal chlamydial dose, <0.25 μg/ml. Clindamycin yielded similar results with each strain tested. The minimal inhibitory concentrations ranged from 0.5 to 1 μg/ml, and the minimal chlamydicidal doses ranged from 2 to 16 μg/ml.

Erythromycin, on the other hand, did not yield identical results with all strains. Although most strains were quite susceptible, yielding results analogous to those of tetracycline, two of the isolates (one from the conjunctiva of an infant and the other from an epidemiologically unrelated cervical infection) were less susceptible. Both of these isolates required minimal inhibitory concentrations of >0.5 μg/ml and minimal chlamydicidal doses of ≥1 μg/ml. Some chlamydial replication still occurred at a concentration of 1 μg/ml.

A titration of one of these relatively resistant strains (2 DE) is shown in Table 2. Rosaramicin, another macrolide antibiotic, was effective against these isolates. All strains tested lost viability at ≤0.25 μg of rosaramicin per ml. Interestingly, the relative susceptibilities to these two macrolide antibiotics were not parallel. Isolate 2 DE, one of the two strains relatively resistant to erythromycin, was the most susceptible of the six strains to rosaramicin.

On a weight basis, clindamycin was the least active antibiotic, requiring a concentration of approximately 1 μg/ml for inhibitory activity and up to 16 μg/ml for a chlamydicidal effect. In contrast, rifampin was the most active antibiotic, with a loss of chlamydial infectivity at <0.008 μg/ml for all isolates.

TABLE 1. *Antichlamydial activity of antibiotics against six recent C. trachomatis isolates*

Antibiotic	concn (μg/ml)	
	Inhibitory	Chlamydicidal
Tetracycline	0.008–0.015	0.03–0.125
Erythromycin	0.125–≥1.0	0.5–≥1.0
Rosaramicin	0.015–0.06	0.06–0.25
Rifampin	0.0005–0.002	<0.008
Clindamycin	0.5–1.0	2.0–16.0

TABLE 2. *Inhibition of C. trachomatis isolate 2 DE by various antibiotics*

Concn (μg/ml)	% of inclusions in antibiotic-free controls[a]				
	Tetracycline	Erythromycin	Rosaramicin	Clindamycin	Rifampin
1.0	–	9	—	2	—
0.5	–	62	—	11	—
0.25	–	100	—	67	—
0.125	–	100	—	89	—
0.06	+	100	—	100	—
0.03	3	100	+	100	—
0.016	13	100	3	100	—
0.008	71	100	30	100	—
0.004	100	100	73	100	—
0.002	100	100	100	100	+

[a] –, No inclusions detected in the presence of antibiotic or in blind passage; +, no inclusions detected in the presence of antibiotic, but the passage was positive.

DISCUSSION

Our results confirm that tetracyclines are highly active against *C. trachomatis*. Resistance to this drug has not been found, even when isolates have been obtained from treatment failures (after the patients have been treated with topical tetracyclines). Rifampin was still the most active drug in vitro.

Clindamycin was tested because it is a drug often used in treatment of pelvic inflammatory disease and because its antichlamydial activity has been noted (1). Chlamydiae have been recognized as causes of some cases of acute salpingitis. The organism was shown to be suppressed or killed by concentrations (8 to 16 μg/ml) that are routinely achieved during clindamycin administration.

Erythromycin susceptibility results obtained with two strains were particularly disconcerting. These two isolates were more resistant than any of the other *C. trachomatis* strains tested in the past. Both showed some growth at 1 μg of erythromycin per ml. Although chlamydial growth was still suppressed considerably by this concentration, there was a marked difference in susceptibility compared with the other strains tested. Generally, one considers 1 to 2 μg/ml to be the blood level of erythromycin or tetracycline reached when treating these infections. Our results appear to predict that this blood level of tetracycline would be highly active against all isolates tested but that erythromycin might not be as effective. However, this is not necessarily true, because our results are based on the inhibition of single growth cycles, and the hallmark of antichlamydial therapy is persistent or long-term treatment. Thus, resistance by these concentrations is probably not clinically relevant,

but it is of particular concern that this relative resistance occurs with erythromycin. This is the drug of choice in treating infants with chlamydial conjunctivitis or pneumonitis. Tetracyclines are contraindicated in this population, and the alternate treatment would have to be sulfonamides. Rosaramicin, another macrolide antibiotic, was effective, but it is not yet commercially available. Other workers have found that this antibiotic is extremely active against *C. trachomatis* in vitro (6, 9). We were surprised that its antichlamydial effects did not vary along with the action of erythromycin.

The appearance of this relative resistance may reflect the increased use of erythromycin in treating chlamydial infections in women who cannot take tetracycline or who are pregnant and in infants. Alternatively, these two isolates may represent a subpopulation of strains that have long been circulating but have not been screened. We have not yet passed these isolates in the presence of erythromycin to see whether resistance could be increased. If our studies are detecting a trend, the ultimate emergence of erythromycin-resistant strains will be of considerable practical importance.

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LITERATURE CITED

1. **Alexander, E. R., P. Skahen, and K. K. Holmes.** 1977. Antibiotic susceptibility of *Chlamydia trachomatis* in cell culture, p. 223–226. *In* D. Hobson and K. K. Holmes (ed.), Nongonococcal urethritis and related infections. American Society for Microbiology, Washington, D.C.
2. **Becker, Y., Y. Asher, N. Himmel, and Z. Zakay-Rones.** 1969. Rifampicin inhibition of trachoma agent in vivo. Nature (London) **224**:33–34.
3. **Blackman, H. J., C. Yoneda, C. R. Dawson, and J. Schachter.** 1977. Antibiotic susceptibility of *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **12**: 673–677.
4. **Bowie, W. R., C. K. Lee, and E. R. Alexander.** 1978. Prediction of efficacy of antimicrobial agents in treatment of infections due to *Chlamydia trachomatis*. J. Infect. Dis. **136**:655–659.
5. **Keshishyan, H., L. Hanna, and E. Jawetz.** 1973. Emergence of rifampin-resistance in *Chlamydia trachomatis*. Nature (London) **233**:173–174.
6. **Kuo, C.-C., S.-P. Wang, and J. T. Grayston.** 1977. Antimicrobial activity of several antibiotics and a sulfonamide against *Chlamydia trachomatis* organisms in cell culture. Antimicrob. Agents Chemother. **12**:80–83.
7. **Ridgway, G. L., J. M. Owen, and J. D. Oriel.** 1976. A method for testing the antibiotic susceptibility of *Chlamydia trachomatis* in a cell culture system. J. Antimicrob. Chemother. **2**:71–76.
8. **Schachter, J.** 1978. Chlamydial infections. N. Engl. J. Med. **298**:428–435, 490–495, 540–549.
9. **Smith, T. F., and H. E. Washton.** 1978. In vitro susceptibility of 30 strains of *Chlamydia trachomatis* to rosamicin. Antimicrob. Agents Chemother. **14**:493–494.
10. **Wang, S.-P., and J. T. Grayston.** 1970. Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test. Am. J. Ophthalmol. **70**:367–374.